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ASSESSING THE EFFECTS OF SOIL MOISTURE ON GERMINATION OF WINTER
WHEAT

By
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Under the Supervision of Dr. P. Stephen Baenziger and Dr. Martha Mamo

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ASSESSING THE EFFECTS OF SOIL MOISTURE ON GERMINATION IN WINTER WHEAT

Ellie Smith-Eskridge, B.S.

University of Nebraska, 2014

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Abstract

Drought is a major issue in wheat production globally and in Nebraska particularly during germination. Rapid germination is important for satisfactory stands whereas late germination may lead to a lower yield. Laboratory experiments were conducted at the University of Nebraska-Lincoln (UNL) to compare germination of five Nebraska-grown wheat varieties, or cultivars, under water stress conditions.

The cultivars, Anton, Nuplains, Pronghorn, Trego, and Wesley were selected because of their presumed differing water use efficiency (WUE). Tests were conducted on a Nebraska soil to create a soil water release curve and to select soil moisture level or water treatments. The experimental design was a 3X5 factorial consisting of three water treatments and 5 cultivars replicated four times. Seeds with the appropriate water treatments were incubated at 21° C and germination success was measured on the fifth and twelfth day after incubation. Analysis of variance was used for data analyses and significance tests. Germination differed significantly across cultivars and water treatments. A significant interaction was found between cultivars and water treatment ($p < 0.05$). Anton followed by Wesley

showed the highest germination under the highest water stress condition (8 ml). Anton may be a more drought tolerant cultivar but it has a low tolerance to pre harvest sprouting in humid conditions, whereas Wesley may be the best cultivar among those tested because of its high germination under water stress conditions and high tolerance to pre harvest sprouting. This experimental approach is an inexpensive screening method that can be used to help select drought tolerant wheat genotypes. Since drought may become more prevalent and wheat is often grown on soils that have a low water holding capacity, this research can lead to the development of drought tolerant cultivars in order to feed an increasing global population.

Preface (Acknowledgements)

I would like to thank my thesis advisors, Dr. P. Stephen Baenziger and Martha Mamo for providing me guidance throughout my research, Dr. Kent Eskridge, Gen Li, and my environmental studies advisors, Dr. Dave Gosselin and Sara Cooper for being supportive and helpful, and the Undergraduate Creative Activities Research Experiences (UCARE) for providing the research funding.

Introduction

Wheat feeds one third of the world population and supplies more than half of their calories and nearly half of their protein (Dhanda et al., 2004). Also, it is one of the most essential sources of fiber in the human diet, and as people become wealthier, they tend to eat more wheat and wheat-based products. Wheat is consumed globally in countries of primary production as well as countries where wheat cannot be grown (Shewry, 2009). The U.S. is one of the most important wheat producing countries and produces wheat for both the national market as well as export. Nebraska is one of the major wheat producing states in the U.S found in the “breadbasket” of the world (Moore and Rojstaczer, 2001).

Drought in Western Nebraska is a major environmental and agricultural issue and continues to affect wheat and grain production. Approximately 75% of wheat in Nebraska is grown in the western region where about 92% of winter wheat acreage is in dry land production without irrigation or supplemental water (Hamid, 2012). Soil with low water holding capacity causes stress on plants during drought. Soils especially in Western Nebraska generally have low water holding capacity, and low soil water coupled with dry land production results in high risk for crop failure during drought (Wilhemi and Wilhite, 2002).

Climate models have predicted an overall global increase in severe droughts during the 21st century including North America (Dai, 2010). As climate changes, Nebraska may be prone to more droughts, and adaptations to future increased aridity should be considered (Dai, 2010).

Drought can have drastic effects on wheat during various periods of plant development, particularly at germination. At this stage of emergence, crop establishment is critical for producing a sufficient stand, which will produce an adequate crop if the subsequent growing conditions are ideal for growth and development. Thus, in order to screen wheat varieties, or cultivars that are more drought tolerant, determining the rate of germination and emergence under different soil moisture levels are essential. Lindstrom et al. (1976) discuss that rapid emergence of winter wheat is important for optimum fall growth and satisfactory stands of wheat crops. In contrast, later establishment of the crop in the fall due to late germination may lead to spotty stands and eventually to a lower yield. Soil water potential or the amount of water available in the soil also controls the rate of germination and emergence. When the soil moisture content is low or has low soil water potential, the rate of germination is delayed, seeds may become infected with pathogens, and the stands will be thin with weakened plants. However, wheat is one of the few plants that can still germinate at a sufficient rate in conditions of soil water potential that can be lower than -1500 kPa (Lindstrom et. al, 1976).

One strategy to determine drought tolerant cultivars at emergence is to evaluate wheat genotype germination at low soil moisture levels that would be found in semi-arid soils. Genotypes differ in their ability to germinate at low soil moisture contents. Hence, these genotypes have added value in arid conditions. This research is particularly pertinent, as in 2012 there was insufficient water for the wheat-breeding nursery to germinate at North Platte, NE. According to Rhajaram et

al., (2004) certain cultivars have performed well under drought conditions grown in regions of Central Asia, where precipitation is extremely variable. Studies have found that these cultivars contain a drought adaptation gene (Rhajaram, 2004). Perhaps cultivars in Nebraska could also potentially contain this genotype.

The purpose of this experiment is to compare germination among five cultivars under water stress conditions. Cultivars found to have the ability to germinate under water stress will adapt and survive under drought conditions at planting, which occurs periodically in Nebraska. By determining the most water-use efficient cultivars under drought conditions, we will be able to conduct further research on genes regulating low moisture germination. Finally, cultivars with the highest water use efficiency (WUE) will aid farmers and plant geneticists to identify the most water efficient and drought tolerant cultivars for production or research.

Materials and Methods

Soil Water Energy Curve and Water Treatment Selection:

A Soil moisture curve was determined for a silt loam soil (43.8% sand, 41.2% silt, and 15% clay) following standard procedure (Klute, 1986). The relation between soil moisture tension (kPa) and soil moisture content (g of water per g of soil) is called a moisture retention curve or soil moisture characteristic or energy curve (Brady and Weil, 2010). In order to construct the moisture retention curve of the soil and derive the water treatments for the germination test, the moisture content of the sample was measured by setting the near saturated soil at a succession of known water potentials, namely -10, -33, -50, -100, -500, -1500 KPa, until equilibrium was attained at each (Figure 1). The gravimetric soil moisture retained after equilibrium was then determined from a subsample following equation 1 below. The soil water contents lower than -1500 kPa were estimated using the soil texture and the Saxton model (Saxton et al., 2004).

Water content by mass (g water/g soil) = (Moist Soil -Dry Soil)/Dry soil [Eq. 1]

For this experiment, drier soil conditions representing lower soil water potentials below the wilting point of -1500 kPa were selected. Experimental water potentials were -1700 kPa, -1800 kPa, and -1900 kPa, which corresponded to water treatments of 10, 9, and 8 ml, respectively. Based on preliminary tests, water treatments, -1900 kPa and -1700 kPa, were selected because there was more germination at -1700 kPa and less germination at -1900 kPa. The middle water

treatment of -1800 kPa was selected to observe the responses of germination between -1700 kPa and -1900 kPa.

Germination Test:

Germination tests were conducted to evaluate two factors: water treatments and cultivars. Five different cultivars were selected with diverse genetics and presumed water use efficiency (WUE) determined at maturity by previous experiments. Each cultivar was tested at three water treatments of either 8 ml, 9 ml, or 10 ml (30 treatments). Fifteen petri dishes each representing one experimental unit from the 3x5 factorial design were placed in the incubator. This was followed a total of four times producing four replications of 120 experimental units.

Each of the water treatment and 70 g of soil were mixed for one to two minutes before being placed into the petri dishes. The amount of water added was adjusted to account for the water absorbed by each seed (17 mg per seed) (Wuest and Lutcher, 2012) (Table 1). One piece of filter paper was set on top of the soil and seeds were placed on top of the filter paper in order to see the development and to avoid interference with germination (Wuest, 2007). Twenty dried seeds were then placed on the filter paper, covered with the lid, and incubated at 21 °C. Light was turned on in the incubator for eight hours per day. The petri dishes were placed lid side down in the incubator in order for the radical to grow towards the lid and easier to see (Wuest, 2007). Seeds were grown and observed for up to 25 days (Wuest and Lutcher, 2013). However, as wheat normally germinates in three to four days, many of the treatments were fully germinated and scored before 14 days.

Germination was scored on the fifth day and twelfth day after watering and germination scoring ended on the twenty-fifth day. When a 5-millimeter root or shoot sprouted from the seed, it was considered as germinated (Wuest and Lutchy, 2013). The total number of seeds germinated per petri dish by the end of the experiment was used to measure germination. This indicated if the rate of germination was affected by the water and cultivar treatments. Germination percentage out of 20 seeds was calculated for each petri dish experimental unit.

The germination percentages were statistically analyzed using an analysis of variance and the means compared using multiple range comparisons within and among the water and cultivar treatments. Experimental and control differences were compared across all cultivars to identify which cultivars differed significantly regarding germination. The most and least efficiently germinating cultivars were repeated to ensure our WUE assay was repeatable and capable of separating cultivars.

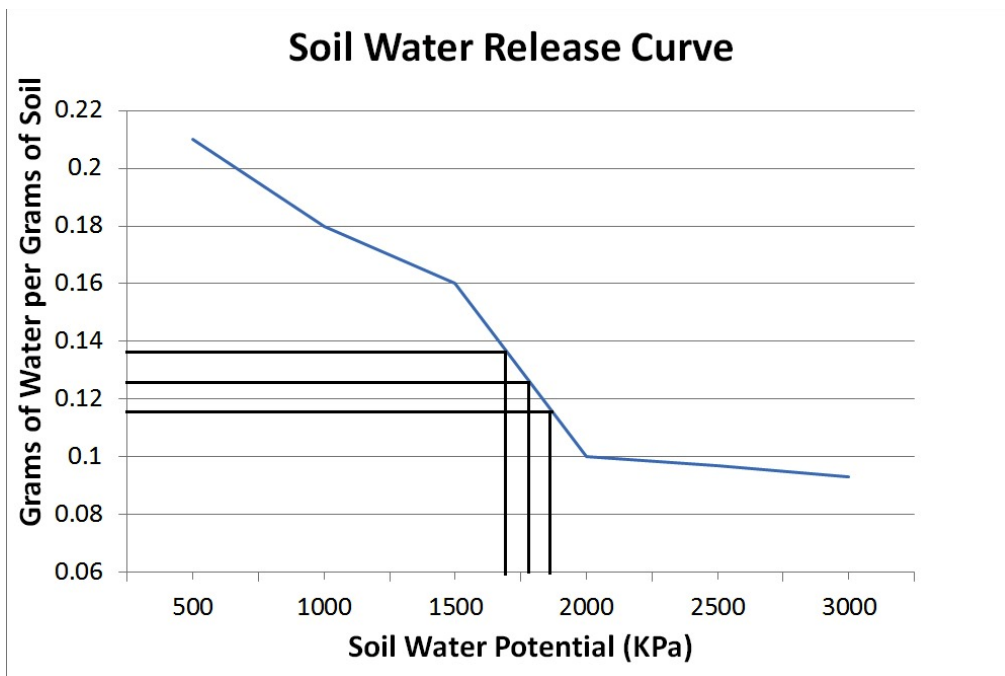


Figure 1. The graph shows the soil water release curve, or soil moisture retention curve, of a silt loam soil, which pinpointed the three soil water potentials and mass water content (g of water/g of soil) under water stress conditions to choose the three water treatments.

Table 1. Soil water tension and corresponding soil water content of the three water treatments used to test germination of five wheat cultivars.

Soil Water Potential (kPa)	Soil Water Content (g water/g soil)	Water Added per 70 g of dry soil (ml)	70 grams of soil + water absorbed by seed (ml)*
-1700	0.137	$0.137 \times 70 = 9.59$	$9.59 + 0.34 = 9.93 \approx 10$
-1800	0.125	$0.125 \times 70 = 8.75$	$8.75 + 0.34 = 9.09 \approx 9.1$
-1900	0.115	$0.115 \times 70 = 8.05$	$8.05 + 0.34 = 8.39 \approx 8$

*Seed water absorption 17 mg/seed.

Results

The germination percentages were significantly different among cultivars ($p < 0.05$) (Table 2). There was a significant effect of water treatment ($p < 0.0001$, 8 ml=24.3 %, 9.1 ml=67.9%, 10 ml=76.8% germination, Table 2) with 8 ml (-1900 kPa) differing from 9.1ml (-1800 kPa) and 10 ml (-1700 kPa) ($p < 0.05$) (Table 1). There was no significant difference in germination percentage between 9.1 ml and 10 ml water treatments ($p > 0.05$). A significant interaction existed between the cultivar and day ($p < 0.05$, Day 5=10%, Day 12 =12.5% germination, Figure 2) but there was not a significant interaction of cultivar by water treatment ($p > 0.05$, Figure 3). In addition, there was significantly higher germination on day twelve than day five (Table 2, Figure 2).

Anton showed the highest mean germination of 74% followed by Wesley with 57% germination, and Nuplains with 53% mean germination. Trego and Pronghorn, showed similar mean germination responses to the three water treatments of approximately 50% and 48% mean germination where Pronghorn had the lowest germination (Table 2).

Anton was the most responsive as the water treatments increased with Anton reaching approximately 48% germination at 8 ml of water and approximately 86% germination at 9.1 and 10 ml of water (Table 2, Figure 3). In addition, Anton showed approximately 71% mean germination on the fifth day and approximately 77% mean germination on the twelfth day (Table 2, Figure 2).

The cultivar, Wesley, showed the second highest germination, and responded similarly to Anton at 9.1 ml of water, but was not as responsive at 10 ml of water.

Wesley reached 16% germination at 8 ml, 82% germination at 9.1 ml, and 73% germination at 10 ml (Table 2, Figure 3). Additionally, Wesley showed an increase in percent germination from the fifth to twelfth day reaching 48% and 67% germination, respectively (Table 2, Figure 2).

The cultivar, NuPlains, was less responsive than Anton and Wesley to the increasing water treatments, and responded most linearly as water treatments increased. NuPlains reached approximately 30% mean germination at 8 ml of water, 54% mean germination at 9.1 ml of water, and 73% mean germination at 10 ml of water (Table 2, Figure 3). NuPlains showed a 45% mean germination on the fifth day and approximately 61% mean germination on the twelfth day (Table 2, Figure 2).

The cultivars, Pronghorn and Trego were similar in response but were not as responsive as the others. Both showed approximately a 14% and 11% mean germination at 8 ml, a 57% and 61% mean percent germination at 9.1 ml, and 76% mean germination at 10 ml of water (Table 2, Figure 3). On the fifth and twelfth day, Pronghorn increased its germination percentage from 45% to 51%, and Trego increased its mean germination percentage from 43% to 57% (Table 2, Figure 2).

Table 2. Mean percent germination for cultivars, water treatments, and days after experimental set up.

Water treatment/ Cultivar	8 ml	9.1 ml	10 ml	Average	Day 5	Day 12
Anton	48.1%	86.9%	86.2%	73.8%	70.8%	76.7%
Nuplains	31.9%	53.8%	72.5%	52.7%	44.6%	60.8%
Pronghorn	13.8%	55.6%	75.6%	48.3%	45.4%	51.3%
Trego	11.3%	61.3%	76.3%	49.6%	42.5%	56.7%
Wesley	16.3%	81.9%	73.1%	57.1%	47.5%	66.7%
Average	24.3%	67.9%	76.8%		50.2%	62.4%

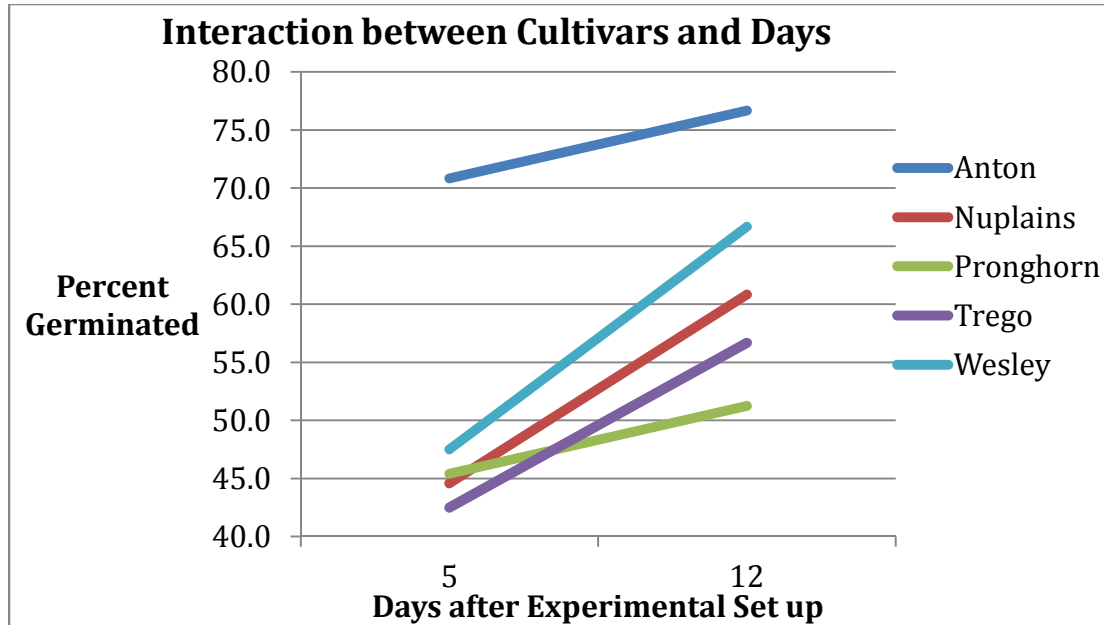


Figure 2. The graph depicts a significant interaction between cultivar by day after experimental set up ($p < 0.05$).

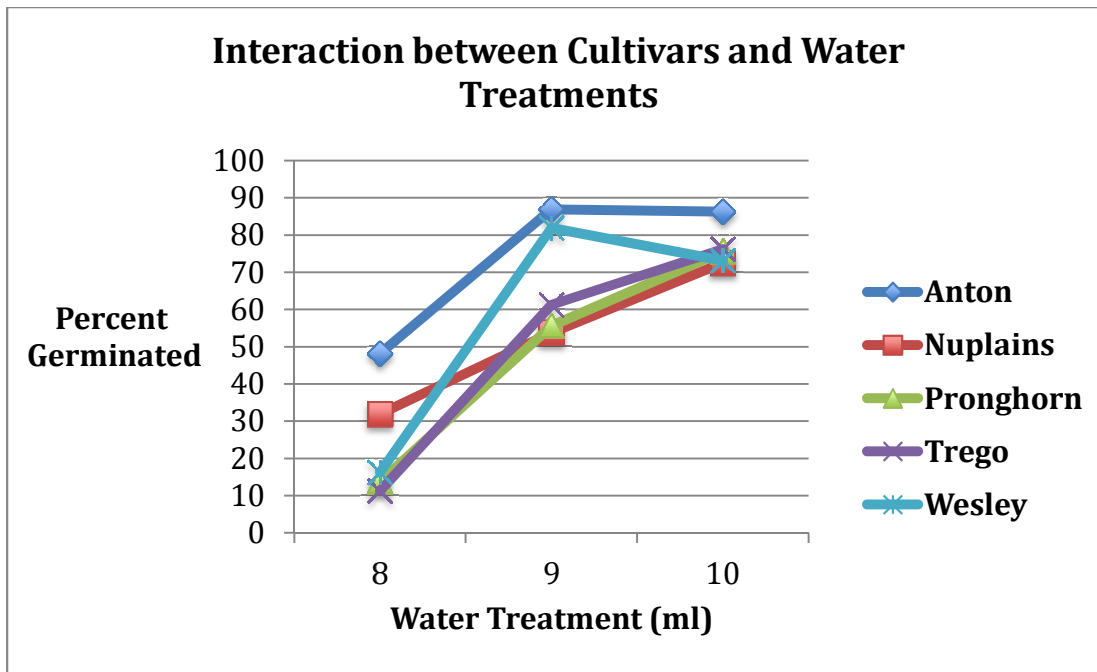


Figure 3. The graph represents an interaction of cultivar by water treatment, which was not significant ($p > 0.05$).

Discussion

It was found that germination of cultivars under water stress conditions significantly differed ($p < 0.05$). Anton differed from many of the cultivars and was the most responsive regarding germination under water stress conditions followed by Wesley. This was clearly seen in Figure 2 where Anton showed much higher mean percent germination followed by Wesley on the fifth and twelfth day, whereas Trego, Nuplains, and Pronghorn were not as responsive. Although the interaction of cultivars by water treatment was not significant ($p > 0.05$), Anton's high response was highest of all cultivars (Figure 3). Therefore, Anton may be a more drought tolerant crop compared to the other cultivars.

Germination was higher in the 10 ml water treatment compared to the 8 ml water treatment, and 9.1 ml water treatment was also higher compared to 8 ml water treatment. However, Figure 3 showed that lower germination was found at 10 ml rather than 9.1 ml especially in Wesley. This result can be explained by experimental variation because 9.1 ml and 10 ml were not significantly different from each other.

An important consideration for interpretation is that pre harvest sprouting (PHS) might be related to early germination (Graybosch et al., 2013). Pre harvest sprouting occurs when seeds within the grain head of the plant germinate before harvest under wet conditions. Unfortunately, this is an issue when cultivars including Anton have low tolerance to pre harvest sprouting which leads to reduction in yield and financial losses to farmers (pubs.ext.vt.edu). Cultivars that germinate more quickly than others may also have a low tolerance to pre harvest

sprouting, and cultivars that are known to germinate rather slowly would have a higher tolerance to pre harvest sprouting. However, according to pre harvest sprouting research, this is not the case. Table 3 shows some cultivars might germinate more slowly from this research, which is different from a cultivar's low tolerance to PHS from previous research.

Since Anton showed significantly higher germination among all cultivars ($p < 0.05$), this is supported by previous work that found Anton to have low tolerance to pre harvest sprouting in humid areas (Graybosch, et al., 2013, Table 3). Hence, Anton may be a better candidate to cultivate under drought conditions rather than in wet conditions.

Wesley had nearly as high germination as Anton, which is different from previous work that found Wesley to be the most tolerant to PHS (Graybosch, R., et al., 2013, Table 3). Perhaps Wesley is more beneficial to cultivate in the field than Anton because it is highly tolerant to PHS and still appears responsive under water stress conditions regarding germination.

Pronghorn was least responsive to water treatments which appears contrary to Pronghorn's low tolerance to pre harvest sprouting (Graybosch, R., et al., 2013, Table 3). Pronghorn's low germination response under water stress conditions was also different from previous work, which found Pronghorn to have one of the highest germination rates (Erayman et al., 2006). Additionally, Trego and Nuplains had moderate levels of germination, which is similar to their moderate tolerance to pre harvest sprouting (KSU, 2000, Table 3).

This research on germination of the five cultivars under water stress conditions may have differed from previous work on PHS because of the different environmental conditions. This experiment was conducted in a lab under water stress conditions whereas the previous research on pre harvest sprouting was followed in wetter conditions and/or in the field where numerous environmental conditions such as temperature and humidity could not be controlled.

Table 3. Germination of cultivars from this research compared to previous work involving PHS tolerance.

Cultivar	Germination	PHS Tolerance	Compared to previous work?
Anton	1 st Highest germination	low	Similar to Graybosch, 2013
Wesley	2 nd highest germination	high	Different from Graybosch, 2013
Pronghorn	Lowest germination	low	Different from Graybosch, 2013
Trego and Nuplains	3 rd and 4 th highest germination	moderate	Similar to KSU, 2000

Summary and Conclusion

The research was conducted to compare germination among five cultivars under water stress conditions, which hopefully can lead to the discovery and development of drought tolerant varieties in Nebraska. Overall, germination percentage of cultivars were significantly different ($p < 0.05$) indicating a variation in genotype response to water stress conditions. Anton showed the highest germination under water stress conditions followed by Wesley, Nuplains, Trego, and Pronghorn.

Although Anton seemed to be the most drought resistant, which would tend to indicate it would be most preferable for drought prone areas, it has also been found to have low tolerance to pre harvest sprouting which reduces yield in more humid years (Graybosch, 2012). Perhaps Anton would be a better variety to cultivate in drier conditions. On the other hand, Wesley also germinated almost as well as Anton under water stress condition, and it has a high tolerance to PHS (Graybosch, 2012). Therefore, Wesley might be a beneficial variety to grow in drought prone areas and where humid conditions are possible. Pronghorn showed the lowest germination under water stress conditions, which was different from previous work that found Pronghorn to have low tolerance to PHS (Graybosch, 2012). Pronghorn may not be an ideal variety to grow in both dry and wet conditions. Trego and Nuplains showed moderate germination between all five cultivars, which is similar to previous work that found both to have a moderate tolerance to PHS (KSU, 2000).

Because no significant difference existed between 9.1 ml and 10 ml, the next potential experiment should utilize only one of these water treatments, the water treatment 8 ml, and a well-watered water treatment not found under water stress. These water treatments can then be used to compare the cultivars' germination response between the control and experimental water treatments. The control can be used to verify that the sample of seeds are alive and are still able to germinate under more humid conditions. In addition, another experimental modification could be to measure the coleoptile length instead of the radicle in order to consider if the seeds have germinated. This may be a more efficient strategy to evaluate germination (Erayman, 2006).

Since few studies have researched the potential genetic differences of Nebraska grown cultivars in terms of germination under water stress, this experimental approach can be used to screen wheat genotypes for drought tolerance in further research. Wheat researchers can then use this method as an inexpensive strategy to select genotypes to be grown in the field that may be more easily established in drought prone conditions.

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